Imperial College Healthcare NHS Trust

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Investigating physiological effects of weight loss on male fertility

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Study management group

Chief Investigator: Dr Channa N. Jayasena

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Study Management: Dr Channa N. Jayasena

Clinical queries

Clinical queries should be directed to Dr. Channa Jayasena who will direct the query to the appropriate person.

Sponsor

Imperial College London is the research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:
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This protocol describes the proposed study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

INTRODUCTION

An increasing number of couples find it difficult to have children i.e. they have reduced reproductive capacity (NICE 2016). During the last few decades, tremendous advances have been made in the treatment of women with reduced reproductive capacity e.g. in vitro fertilization therapy (IVF) and specialized clinics for recurrent miscarriage. However, poor sperm quality is the causative factor in 40% of infertile couples i.e. 'male factor infertility'. There is no therapy proven to increase sperm function in men with reduced reproductive capacity. Consequently, the only therapeutic option for male factor infertility is assisted reproductive technologies (ART) such as IVF and intra-cytoplasmic sperm injection (ICSI) (Human Fertilisation and Embryology Authority 2013); these require daily hormonal injections to stimulate several egg follicles to grow in the female partner. The eggs are collected surgically from the ovaries before fertilized with sperm from the male partner, prior to incubation and re-implantation of embryos to the uterus. Although highly effective, IVF and ICSI are invasive and have uncommon but potentially life-threatening complications for the female partner such as ovarian hyper-stimulation syndrome (OHSS) (RCOG 2016). In 2013, over 20,000 ICSI cycles were performed in the UK for male factor infertility, costing a total of £120M (Human Fertilisation and Embryology Authority 2013). NHS funding for IVF and ICSI is restricted and diminishing. Therefore, there exists an important and unmet need to develop practical and cost-effective first-line therapies for male factor infertility.

Obesity is a common cause of reduced reproductive capacity in men. Obesity impairs fertility through multiple mechanisms, including insulin resistance, and increased conversion of testosterone to oestrogen (Craig et al. 2017). A recent large meta-analysis of over 20 observational studies demonstrates that obesity is significantly associated with poor sperm quality (Sermondade et al. 2013). The major association of obesity with poor sperm function makes weight loss a logical treatment. The most effective treatment for obesity is bariatric surgery, but the acute starvation-like state induced by the surgery inhibits sperm function, making this an inappropriate approach (Witalison et al. 2015). But recent evidence suggests that milder weight loss could improve male fertility. Preliminary data suggest that live birth outcomes following IVF treatment are significantly increased when male partners of obese women voluntarily lose 2-3kg weight when compared with male partners of obese women who chose not to lose weight (Belan 2015). However, to date no prospective controlled study has investigated whether weight loss can improve sperm function in obese men with reduced reproductive capacity. Furthermore, it is unclear why moderate but not severe weight loss improves sperm function in obese men with reduced reproductive capacity.

Results of this study will inform us for the first time whether Caloric Restriction using LED increases sperm quality significantly when compared with standard NHS lifestyle advice in obese men with reduced reproductive capacity. In addition, the study may demonstrate that LED significantly reduces levels of markers associated with cardiovascular and diabetes risk in obese men with reduced reproductive capacity. Results of this study are likely to be adopted by many couples and clinicians immediately, and could have a positive effect on live birth rates in affected couples.

The aim of this study is to determine the optimum level of weight loss to improve sperm quality in obese men. We will then investigate how moderate weight loss may improve reproductive function in obese men.

Finally, we will determine if weight loss improves sperm quality and cardio-metabolic health better than standard weight loss advice in obese men with reduced reproductive capacity.

IMPACT: The current level of NHS expenditure on IVF therapy is unsustainable, and there exists no evidence-backed therapy for improving sperm count in men with reduced reproductive capacity. Weight loss via LED could increase the sperm count in obese men therefore, funding this project will help the NHS develop a simple and cost-effective treatment which could reduce our reliance on IVF as a first-line therapy for affected couples.

STUDY OBJECTIVE

1. **Part 1 Objective:** Use caloric restriction to determine the level of weight loss associated with optimum improvement of sperm concentration in men with weight-related reduced reproductive capacity
2. **Part 2 Objective:** Measure changes in the hypothalamic marker of reproductive stimulation, luteinizing hormone (LH) pulsatility, during caloric restriction in men with weight-related reduced reproductive capacity
3. **Part 3 Objective:** Determine whether targeted caloric restriction significantly improves sperm quality, glycaemic and lipid levels when compared with standard NHS dietary advice, in men with weight-related reduced reproductive capacity
4. **Secondary Objectives:** Quality of life of participants undergoing weight loss; pregnancies or live births following participation.

STUDY DESIGN AND PARTICIPANT ENTRY

Study design:

Parts 1, 2 and 3: Physiological, randomized, controlled study using a behavioural intervention

Participant recruitment:

Participants will be recruited whilst attending Medicine or Gynaecology clinics at Imperial College Healthcare NHS Trust hospitals or Patient Identification Centers. Patient records will be assessed and reviewed by members of the direct care team, rather than the research team. They will be informed about the study by a member of the research team who may not be part of their direct care but acting under arrangements with NIHR, or their NHS clinicians. If interested, they will be given a participant information sheet about the study. Paper or online adverts will also be used to enhance recruitment. Potential participants who will respond to paper or online adverts will be informed about the study via phone or email by a member of the research team and if interested, given a participant information sheet about the study via post or letter email. Patients or potential participants via adverts interested to participate, with permission will be briefly screened against the inclusion criteria by a member of the research team. If they broadly fit the criteria of the study, they will be contacted over a period of 24 hours by a member of the research team via post or letter email, who will invite them to attend the Andrology Department, Imperial College Healthcare NHS Trust later for a screening visit during which informed consent will be obtained.

Participants will be asked to provide their GP contact details, so that GPs can be notified by a standard letter. GPs will be given contact details of study investigators in case they have further questions.

Inclusion Criteria for Part 1, 2 and 3

4. Male 18-60 years of age
5. Body mass index (BMI) >25kg/m2
6. Evidence of reduced reproductive capacity (e.g. reduced sperm concentration or motility)

Exclusion Criteria for Part 1, 2 and 3

History of undescended testes, testicular surgery or mumps infection
Hormonal therapy such as testosterone or selective oestrogen receptor modulators
History of systemic cytotoxic therapy or pelvic radiotherapy
Chronic systemic disease, such as cardiac, renal or liver failure
At least one of the following:
- Alcohol intake >30 units per week
- Smoking daily
- Recreational drug use at a frequency not less than weekly

Acute illness likely to affect the result of study
Impaired ability to provide full consent to take part in the study
An occupation requiring strenuous physical exercise that may require a high energy diet

Additional Exclusion Criteria for Part 3:

Female partner aged < age 38 years (to minimize the chance of poor ovarian function)
Infertility in female partner

Withdrawal criteria

The participants have the right to withdraw from the study at any time without needing to provide any reason to the study investigators

METHODS

Confidentiality of participants will be protected as each one will be given an arbitrary unique ID number by a member of the research team. Participants will be randomized using an online randomisation tool, such as 'random.org'.

Part 1: Participants will be randomized to receive standard NHS advice for weight loss, or one of three levels of caloric restriction (e.g. 1500, 1000 and 800kcal/day) during an up to 8-week protocol. 15 participants will be allocated in each of the dietary groups, so 60 participants in total. In participants randomized to one of the energy deficit groups, Cambridge Diet Plan products (approximately 200kcal per product), will be used alone or in combination with normal meals to achieve the desired level of caloric intake. During each study visit, body weight will be measured and body composition will be assessed using bioelectrical impedance analysis. Participants will be asked to produce a semen sample and have their bloods taken by an experienced member of the research team, during each study visit within Department of Andrology, Hammersmith Hospital.

Part 2: Participants will be randomized in two groups for a period up to 8 weeks. 12 participants will be allocated per group, so 24 participants in total. One group will receive standard NHS advice (control group) during their study visits. The other group will receive LED during the whole protocol. The level of Caloric Restriction will be based on the results of Part 1. Frequent blood sampling at least every 10min for up to 8h will be performed up to three times during the study to assess pulsatile hormone secretion such as LH pulse frequency, LH secretory mass and pulsatile LH secretion. Frequent blood sampling will be done with insertion of a cannula by a member of the reasearch team which will be removed after the 8h period. Blood will be collected during the morning from fasted participants for various hormone levels, such as reproductive hormones and leptin measurement.

Part 3: Participants actively trying to conceive will be randomised to one of two groups, for a period up to 20 weeks and will receive LED or standard NHS advice for weight loss. 26 participants will be allocated per group, so 52 participants in total. The level of Caloric Restriction will be based on the results of Part 1. Participants will have their semen analysed and bloods taken during each visit by a member of the reasearch team. They will also complete a validated questionnaire at the beginning of and end of study period to assess the effect of weight loss on their quality of life. Lastly, participants will be contacted by a member of the research team up to 24 months following commencement of the study protocol, to ask if they have experienced pregnancy since they were last seen.

SCHEDULE OF STUDY VISITS

Screening visit (Visit 0;(Part 1, 2 and 3):

Consent: The participant will be greeted by a member of the research team or their NHS clinicians and will be shown to a private consultation room. The researcher would then give the participant a brief verbal explanation of the study, answer any questions about the study, and then seek informed, written consent.

Clinical History at Study Recruitment: A brief history will be taken from the patient, including past medical history, drug history, family history, and reproductive history.

Examination: Height and weight will be measured. Testicular volume estimation will be performed using a Praeder orchidometer by a member of the reaseach team. This is a simple and painless procedure which is routinely performed in men with infertility. In brief, the testicular size is compared to a range of plastic or wooden beads of known volume. This procedure usually takes up to 1 min to perform.

Collection of blood and semen: Venepuncture will be performed by a member of the research team to collect a blood sample (maximum volume 20ml) to measure various hormones such as luteinising hormone or testosterone. Blood hormone analyses will be performed by Department of Clinical Biochemistry, Imperial College Healthcare NHS Trust in collaboration with Dr. Emma Walker (Consultant Clinical Biochemist). All participants will be asked to collect their own semen into a sterile container in a designated private room in the Andrology Department, Imperial College Healthcare NHS Trust. In case a participant is unable to provide a semen sample on the day a new appointment will be offered. Participants will be given sufficient time to produce a sample. Semen samples will be analysed for various molecular markers of sperm function such as sperm concentration, sperm motility and reactive oxygen species. Semen analysis will be performed according to WHO criteria for the examination and processing of human semen.

All samples will be anonymised with a unique study code, and stored in Andrology Department, Imperial College Healthcare NHS Trust. Samples will be analysed in Imperial College Healthcare NHS Trust and disposed after the study has finished.

Study visits 1-7 (Part 1): Participants will each undergo an up to 8-week protocol and will be asked to attend up to 8 visits (Screening visit + Visits 1-7) during the study period. Visits would involve brief history, weight measurement, blood pressure, body composition analysis and advice on total daily caloric intake. Blood and

semen will be additionally collected as outlined in the screening visit. This would take 30 to 60min to perform.

Study visits 1-7 (Part 2): As described above, half of patients will receive unrestricted caloric intake and the other half LED during the whole protocol. Obese men with infertility will each undergo an up to 8-week protocol and will be asked to attend up to 8 visits during the study period. Frequent blood sampling every at least 10min for up to 8h will be performed at the beginning of and end of study period at the final week to assess hypothalamic function in response to weight loss. Frequent blood sampling will be performed during the 1st visit which will be in weeks 1 or 2, 2nd frequent blood sampling visit will be in weeks 3 or 5, and 3rd frequent blood sampling visit during final 2 weeks.

Study visits 1-7 (Part 3): Participants will each undergo a maximum of 20-week protocol with up to 8 study visits. The protocol consists of the following phases: an initial phase of baseline observation within a period of up to 4 weeks; a second period of randomisation up to 8 weeks, when half of participants will receive NHS lifestyle intervention and the other half LED; a final period of up to 8 weeks when both groups will undergo post-treatment observation. During each study visit from baseline observation to the period of randomisation as well as post-treatment observation period, body weight and blood pressure will be measured. Body composition will be assessed using bioelectrical impedance analysis. Participants will be asked to produce a semen sample during each study visit within Department of Andrology, Hammersmith Hospital. Semen analysis will measure different parameters such as seminal volume, sperm concentration, total and progressive sperm motility, and sperm morphology, reactive oxygen species and sperm DNA fragmentation index. During each visit, blood will be collected during the morning from fasted participants for different hormones and metabolic parameters such as testosterone, glucose and cholesterol levels. Finally, we will study the effects of weight loss on the quality of life via a validated questionnaire, such as the 'Moorehead-Ardelt Quality of Life Questionnaire II'. We will contact participants via phone or email up to 24 months following commencement of the study protocol, to ask if they have experienced pregnancy since they were last seen.

Contact of participants with fertility problems which have not been diagnosed previously:
If tests performed during the study highlight clinically significant infertility issues which have not been highlighted previously, the study team would facilitate appropriate NHS treatment, by providing any information needed for their GP to make a referral for specialist clinical care.

Statistical considerations:

Sample size: *The sample size calculations have undergone national, external peer review by the Fellowships committee of the National Institute for Health Research (NIHR). Calculations were performed by an independent statistician (Dr. Les Huson, Senior Statistician, NIHR Imperial Clinical Research Facility), and used assumptions based on clinical experience including anticipated participant drop-outs.*

We will use mean levels of sperm concentration during the study period as a primary endpoint, which should have less variability in levels when compared with sperm concentration during a single study visit alone.

Part 1: Under the assumption that the mean final sperm concentration in the control group will be 2 mill/ml, and that, in the group having maximum calorie deficit the mean final sperm concentration will be 12 mill/ml (a 6-fold increase), 12 subjects in each of the dietary groups will give greater than 90% power to detect a statistically significant linear trend in increased sperm concentration across the groups [Pinheiro J Biopharm Stat 2006]. This calculation assumes that the between-patient standard deviation in sperm concentration is 6 mill/ml, which is a conservative estimate based on doubling the standard deviation observed in a sample

of data from 1000 patients treated in the Andrology Department at Hammersmith Hospital. Note also that the sample size of 12 per group matches the recommendation made by Julious [Pharm Stat 2005] for pilot and exploratory studies in cases where there is uncertainty about effect sizes. Based on previous experience, we anticipate a 20% drop-out rate, so will recruit 15 participants per group i.e. 60 participants in total.

Part 2: Based on published data, LH pulse amplitude has a mean 3.8+/-0.9iU/L [Spijkstra JJ et al. J Clin Endocrinol Metab 1988]. A sample size of 10 per group would have 80% power detecting a 30% biologically meaningful change in LH pulse amplitude, assuming 5% significance. Based on previous experience, we anticipate a 20% drop-out rate, so will recruit 12 participants per group i.e. 24 participants in total.

Part 3: On the conservative assumption that NHS dietary advice under research conditions will increase sperm concentration from 2 to 6mill/ml (3-fold), which is half of the 6-fold effect size anticipated during LED (see Study 1 analysis plan), then 22 per group would give 90% power to detect a statistically significant increased sperm concentration between groups (alpha=0.05, two-sided). This calculation assumes that the standard deviation in sperm concentration is 6 mill/ml, which is a conservative estimate based on doubling the standard deviation observed in a sample of data from 1000 patients treated in the Andrology Department at Hammersmith Hospital. Based on previous experience, we anticipate a 20% drop-out rate, so will recruit 26 participants per group i.e. 52 participants in total.

Figure 1. Summary of study 1 protocol

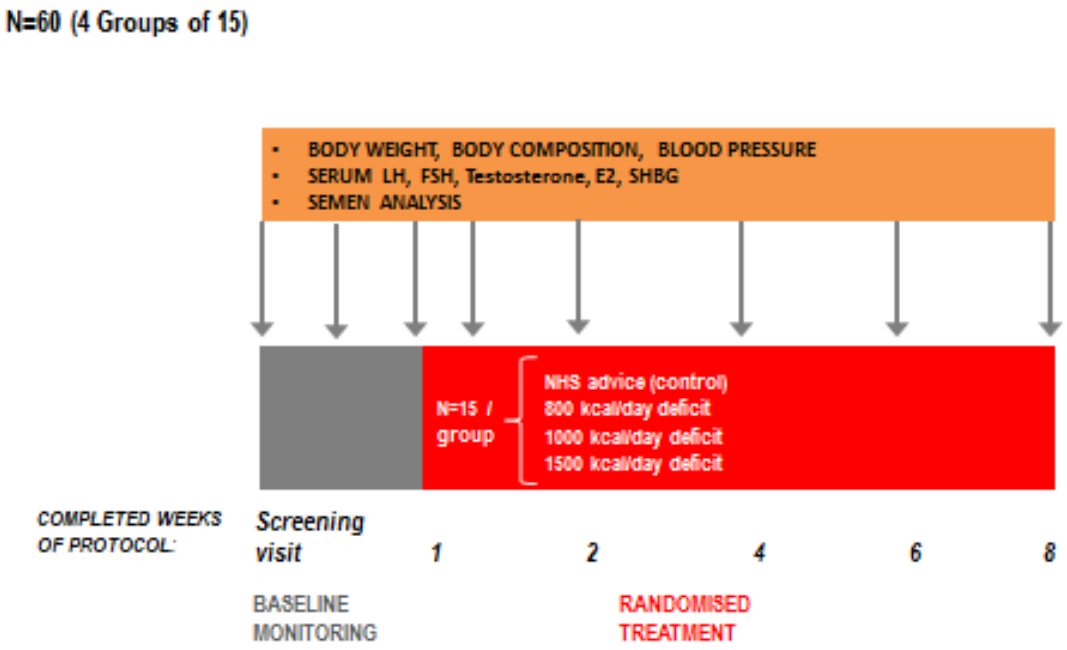


Figure 2. Summary of study 2 protocol

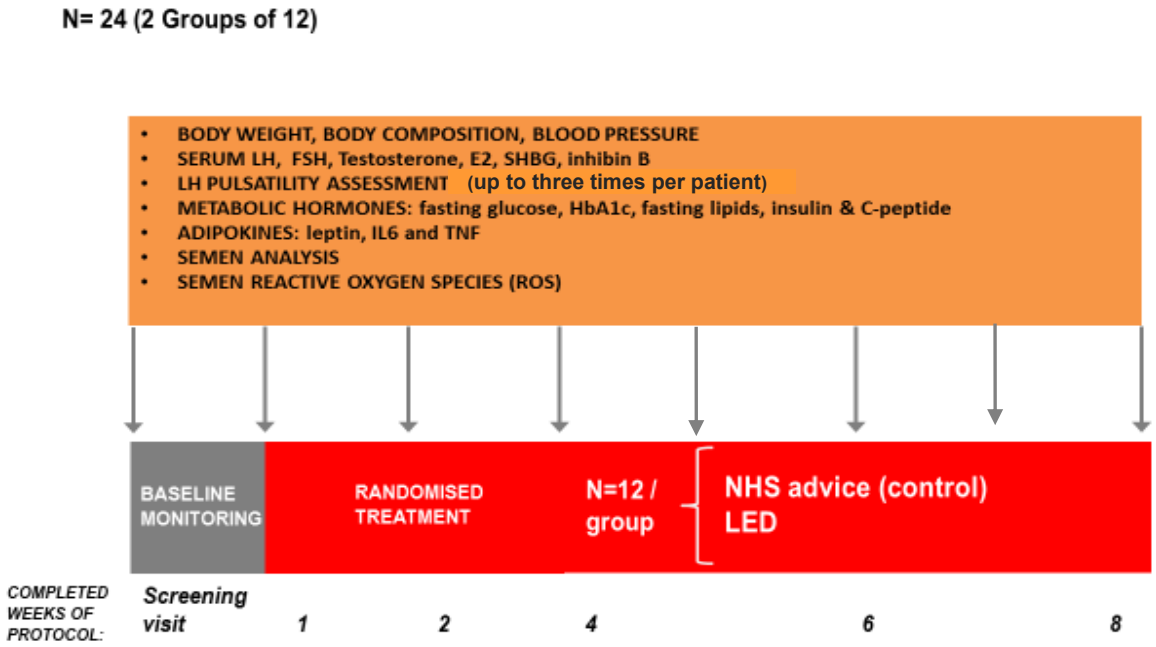
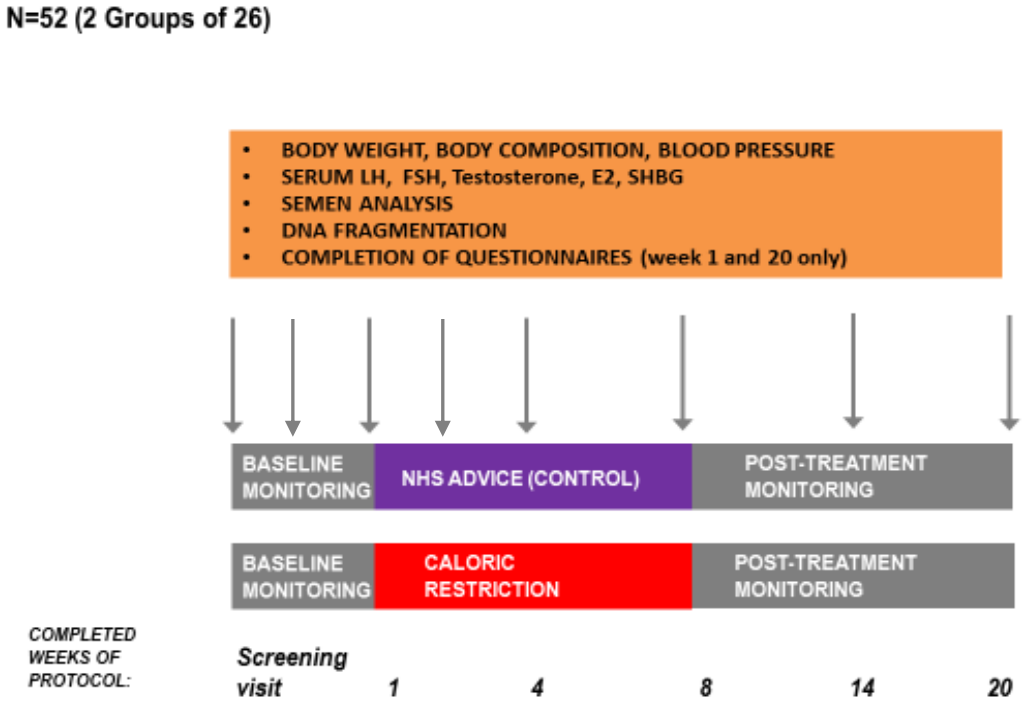


Figure 3. Summary of study 3 protocol



SAFETY AND PROTECTION OF PARTICIPANTS

Venepuncture may cause mild pain or discomfort. Some inconvenience and embarrassment to participants may result from answering questions about their health, providing semen samples and undergoing testicular volume assessment. This will be explained to participants in the information sheet prior to consent being sought.

To minimise any risks, inconvenience or embarrassment to participants, the study will be performed by a clinician or allied healthcare professional experienced in performing the above procedures. Participants will have phone or e-mail access to a study investigator during the study to raise any concerns or issues relating to their involvement.

As part of the study some participants will be given LED in the form of liquid products to take for a maximum of 2 months. These are commercially available and considered highly safe, so is not anticipated to have associated risks. Lifestyle advice should be given to all patients as part of routine primary care, so we do not anticipate randomization to the simple lifestyle advice group to unduly disadvantage the healthcare of patients.

ADVERSE EVENTS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity

- Is a congenital anomaly or birth defect

Medical judgment will be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above, will also be considered serious.

Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below will be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs: All such events, whether expected or not, will be recorded.

Serious AEs: An SAE form will be completed and faxed to the Dr. Channa Jayasena within 24 hours. However, hospitalisation, serious illness or death due to unrelated pre-existing conditions, and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the London Bridge REC committee where in the opinion of the Chief Investigator, Dr. Channa Jayasena, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs will be submitted within 15 days of the Chief Investigator, becoming aware of the event. Dr. Channa Jayasena will also notify the Sponsor of all SAEs.

STATISTICS AND DATA ANALYSIS

The analysis plan was made with Dr. Les Huson, Senior Statistician, NIHR Imperial Clinical Research Facility. Currently planned are the analyses below:

- Part 1: Fertility, endocrine and metabolic markers in major groups with different caloric deficit will be compared using one-way ANOVA.
- Part 2: Hormone profiles during 8-hour studies were analysed using ANOVA.
- Part 3: We anticipate that data will be normally distributed. Parameters will be compared in the LED group and group on lifestyle advice using an unpaired two-tailed t-test or equivalent non-parametric tests if required
- Changes in fertility markers will be correlated with endocrine markers (e.g. Testosterone) using linear regression.
- We also anticipate distributing questionnaires in a maximum of 52 patients. Qualitative analysis methods will be utilised to provide a summary of the participants answers to the questionnaire.

REGULATORY ISSUES

Ethics approval

The Chief Investigator, Dr. Channa Jayasena, has sought approval from the XXXXX. The study will be submitted for Health Research Authority Approval (HRA) at Imperial College Healthcare NHS Trust. The Chief Investigator will obtain confirmation of capacity and capability approval from the Joint Research Compliance Office before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Consent

Patients attending clinic appointments at Imperial College Healthcare NHS Trust will be informed by a member of staff in the department (clinician, nurse, clinical scientist or receptionist) that a research study is being performed in the department, and they will be given a participant information sheet to explain aspects

of the study. If the patient would like to participate, they will be given an appointment to attend the Andrology Department at Hammersmith Hospital at a later date. On arrival to the Andrology department, a member of the research team researcher would then give the patient a brief verbal explanation of the study, answer any questions about the study, and then obtain informed, written consent if the patient was still happy with give this.

Data handling, record keeping and sample storage

Results of the tests performed during this study could result in incidental findings which are clinically significant (i.e. need further medical assessment). Personal contact details are therefore required for communication with the patients participating in the study. This information is held solely for communication between researchers and participants.

Data security measures are in place at all times according to Imperial College's Information Security Policy. Consent forms containing personal data in paper form will be held in the study master file, which will be kept in a locked office cabinet within a locked office within the Section of Investigative Medicine, Commonwealth Building, Imperial College Faculty of Medicine, Hammersmith Hospital. Technical measures, for example use of encryption tools will be used to protect personal data held in electronic form. This data will be accessible to study investigators.

Personal details stored on NHS computers will be password-protected, and only accessed by researchers involved in the study. Participants will be given an arbitrary personal study code number which will be used throughout the study and in data analysis.

Data will be stored for 10 years according to Imperial College Data Management policy. Processing of personal data including its collection, use, storage, adaptation, dissemination or disposal will follow the principles as set out in the Data Protection Act 1998.

Indemnity

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Sponsor

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

Funding

This is a National Institute for Health Research post-doctoral Fellowship funded project.

Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

STUDY MANAGEMENT

The day-to-day management of the study will be coordinated through Dr Channa Jayasena.

PUBLICATION POLICY

We aim to disseminate data generated during the study via publication in peer reviewed medical journals, presentation at conferences and publication in the lay press as appropriate. During publication the data will be completely anonymised and no personal information will be published. Participant confidentiality will be maintained throughout.

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